Executive summary

The present application seeks to amend Schedule 18—Processing aids of the Australia New Zealand Food Standards Code (the Code) to approve a transglutaminase enzyme preparation produced by Novozymes.

Proposed change to Australia New Zealand Food Standards Code – Schedule 18—Processing aids

Schedule 18—Processing aids is proposed to be amended to include a genetically modified strain of *Bacillus licheniformis* expressing a transglutaminase from *Streptomyces mobaraensis* as permitted source for transglutaminase.

The application is applied for assessment by the general procedure.

Description of enzyme preparation

The enzyme is a protein-glutamine γ-glutamyltransferase (EC 2.3.2.13), commonly known as transglutaminase.

Transglutaminase catalyses the hydrolysis of the formation of an isopeptide bond between γ-carboxamide groups of glutamine residue side chains and the ε-amino groups of lysine residue side chains with subsequent release of ammonia.

The enzyme is produced by submerged fermentation of a *Bacillus licheniformis* microorganism expressing a transglutaminase from *Streptomyces mobaraensis*.

The transglutaminase enzyme preparation is available as a liquid preparation complying with the JECFA recommended purity specifications for food-grade enzymes.

The producing microorganism, *Bacillus licheniformis*, is absent from the commercial enzyme product.

Use of the enzyme

The transglutaminase enzyme preparation is used as a processing aid in baking processes, brewing processes, cereal-based processes, cheese production in dairy processing, fermented dairy products and plant processing for production of dairy, egg and meat analogues, processed meat and fish. Generally, transglutaminase catalyses the formation of an isopeptide bond between γ-carboxamide groups of glutamine residue side chains and the ε-amino groups of lysine residue side chains with subsequent release of ammonia.

- during baking processes the transglutaminase helps to form the network by forming cross-links between the proteins present in the dough.
- during brewing processes the transglutaminase can form cross-links between the proteins and peptides thereby increasing their molecular weight. As a result the proteins precipitate and will eventually be removed by filtration.
- during cereal-based processes the transglutaminase degrades forms cross-links between the proteins leading to improved mechanical strength of the dough.

- during cheese production in dairy processing the transglutaminase is added during the coagulation step in order to cross-link milk proteins resulting in a stronger protein network and improved water retaining capacity. This results in a higher yield of the cheese together with improved whiteness and texture.
- during fermented dairy products the transglutaminase can be added before or during acidification where it cross-links milk proteins.
- during plant processing for production of dairy analogues the transglutaminase can be used to produce fermented dairy analogues as well as cheese analogues. In the production of cheese analogues, e.g. tofu, the proteins present in the plant materials are cross-linked by the transglutaminase to form a curd-like network. This is then treated similar to traditional cheese production. During production of plant-based fermented dairy analogues the emulsified plant proteins are treated similar to traditional milk proteins during production for fermented dairy products.
- During plant processing for production of egg substitute the transglutaminase is added to this mixture where it cross-links the proteins in order to give the mixture an egg-like structure.
- During plant processing for production of meat and fish analogues the transglutaminase is added in order to form cross-links between the proteins. After inactivation, the cross-linked proteins are mixed with other ingredients and then extruded to create meat-like structures.
- During processing of meat and fish products, the transglutaminase is added and forms cross-links between the proteins resulting in sausages, patties, fish cakes and other products which are formed and packaged. The food enzyme is inactivated by heat.

Benefits

The benefits of the action of the transglutaminase in baking processes are:

- improved dough strength and mechanical tolerance in the baking process resulting in increased fermentation tolerance during baking which might otherwise be impaired by industrial processing of the dough
- improved structure of dough in low quality flour baking
- improved formation of protein network resulting in increased volume in gluten-free applications
- protects the frozen product from damage during frozen storage

The benefits of the action of the transglutaminase in brewing processes are:

- reduction of haze formation
- improvement of foam volume
- reduction of gluten present in raw materials

The benefits of the action of the transglutaminase in cereal-based processes are:

- improved dough mechanical strength of pasta/noodle dough

- improved textural properties of pasta and noodles
- improved texture of the pasta made with lower grad wheat flour
- improved nutritional and health aspects by:
    - enabling the incorporation of bran into pasta and noodles
    - reducing starch digestibility
    - enabling incorporation of other flour sources with improved nutritional value
- reduced cooking loss (leaching out of starch) of gluten-free pasta and noodle applications
- extended shelf life of instant noodles due to reduced uptake of fat/oil of instant fried noodles that can go rancid during storage
- reduced steaming and rehydration time of instant noodles

The benefits of the action of the transglutaminase in cheese production in dairy processing are:

- Improved separation efficiency and increased yield in cheese manufacture
- Increased yield and improved texture in cheese production
- Reduced syneresis (whey separation) in cheese spreads upon storage
- Enhance the meltability properties of the curd obtained

The benefits of the action of the transglutaminase in fermented dairy products are:

- Reduced syneresis (whey separation) in fermented milk products and cheese spreads upon storage
- Improved separation efficiency and increased yield in concentrated yoghurt and cheese manufacture

The benefits of the action of the transglutaminase in plant processing for production of dairy analogues are:

Fermented dairy analogues

- Solidification of the fermented dairy analogue products, to increase elastic properties of the emulsions, to improve the texture and the mouthfeel of the product as well as its visual appearance.

Cheese analogues

- increased strength of protein cross-linking network
- retention of the shape easier slicing without crumbling

The benefits of the action of transglutaminase in plant processing for production of egg substitute are:

- Improved egg-like structure
- Improved process similar to egg denaturation

The benefits of the action of the transglutaminase in plant processing for production of meat and fish analogues are:

- Improved meat like structure
- Improved juiciness
- Reduced cooking loss

The benefits of the action of the transglutaminase in meat and fish products processing are:

- Sausage production and fish/sea food cake production
    - Improved structure
    - Improved juiciness
    - Reduced cooking loss

Safety evaluation

The safety of the production organism and the enzyme product has been thoroughly assessed:

- The production organism has a long history of safe use as production strain for food-grade enzyme preparations and is known not to produce any toxic metabolites.
- The genetic modifications in the production organism are well-characterised and safe and the recombinant DNA is stably integrated into the production organism and unlikely to pose a safety concern.
- The enzyme preparation complies with international specifications ensuring absence of contamination by toxic substances or noxious microorganisms
- Sequence homology assessment to known allergens and toxins shows that oral intake of the transglutaminase does not pose food allergenic or toxic concern.
- Two mutagenicity studies *in vitro* showed no evidence of genotoxic potential of the enzyme preparation.
- An oral gavage administration study in rats for 13-weeks showed that all dose levels were generally well tolerated and no evidence of toxicity.

Furthermore, the safety of the transglutaminase preparation was confirmed by external expert groups, as follows:

- Denmark: The enzyme preparation was safety assessed resulting in the authorisation of the enzyme product by the Danish Veterinary and Food Administration.

Conclusion

Based on the Novozymes safety evaluation, confirmed by the above-mentioned bodies, we respectfully request the inclusion of the transglutaminase in Schedule 18—Processing aids.

Introduction

The present application describes a transglutaminase enzyme preparation produced by submerged fermentation of a *Bacillus licheniformis* microorganism producing a transglutaminase from *Streptomyces mobaraensis*.

The enzyme is a protein-glutamine γ-glutamyltransferase (EC 2.3.2.13), commonly known as transglutaminase. The enzyme catalyses the hydrolysis of the formation of an isopeptide bond between γ-carboxamide groups of glutamine residue side chains and the ε-amino groups of lysine residue side chains with subsequent release of ammonia.

The transglutaminase enzyme preparation is intended to be used as a processing aid for cross-linking protein glutamine and lysine during processing of protein glutamine and lysine-containing foods. It is used in food manufacturing as a processing aid to ease and optimise the processes and make more efficient use of raw materials. Also, the use of the food enzyme ensures a consistent process and minimises potential end product variations.

The following sections describe in detail the construction of the genetically modified *Bacillus licheniformis* used as the production organism, the production process, the product specification, the application of the enzyme preparation and finally the safety evaluation of the product including the toxicology program, which has been carried out confirming the safety of the product for its intended use.

The documentation has been elaborated according to the Application Handbook from Food Standards Australia New Zealand as of 1 July 2019, applied as relevant for an enzyme application, *i.e.* outlining the following section:

- Section 3.1.1 – General requirements
- Section 3.3.2 – Processing aids, subsections A, C, D, E, F

NB! In Appendix 2.1, the transglutaminase enzyme preparation is described by its commercial name, Galaya Prime.